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FilmArray® respiratory panel performance in respiratory samples from neonatal care units

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ABSTRACT

FilmArray Respiratory Panel (RP) (Idaho Technology, Inc., Salt Lake City, UT, USA) performance was retrospectively evaluated in respiratory samples collected from neonates in 2 reference neonatology units. Using the FilmArray RP assay, 121/152 (79.6%) samples were positive for at least 1 respiratory virus, while 31/152 (20.4%) were negative. FilmArray RP results were concordant in 68/72 (94.4%) respiratory samples tested with laboratory-developed real-time PCR assays, while in 4/72 (5.6%) samples, the FilmArray RP assay detected an additional virus (2 human rhinovirus/enterovirus and 2 bocavirus). In addition, FilmArray RP results for 70 of 80 (87.5%) respiratory samples tested were concordant with the Seegene Seeplex RV15® detection assay (Seegene, Inc., Seoul, South Korea), while 10/80 (12.5%) were discordant. The advantages of the FilmArray RP are the rapid detection of respiratory viruses (1 hour), the wide number of pathogens detectable in a single assay, and the reduced hands-on time.

1. Introduction

Viral respiratory outbreaks in neonatal care units are burdened by high morbidity and mortality, and their management implies high healthcare costs (Civardi et al., 2013; Nair et al., 2013). Several community-acquired viruses are responsible for respiratory infections in newborns that give rise to nosocomial outbreaks (Gelber and Ratner, 2002; Faden et al., 2005; Gagneur et al., 2008). Conventional infection control methods (such as hand hygiene and patient isolation and/or cohorting) are recommended, but adoption of rapid, sensitive, and specific diagnostic tools is mandatory for the management and antiviral treatment of these severe clinical conditions.

The FilmArray Respiratory Panel (RP) (Idaho Technology, Inc., Salt Lake City, UT, USA), which consists of a pouch system with a multiplex PCR test, provides the detection of 18 viruses and 3 bacterial respiratory pathogens in about 1 hour. The system requires only 3–5 minutes of total hands-on time to process 1 sample. In this study, FilmArray RP performance was evaluated in 2 independent laboratories with respiratory samples collected from neonatal patients.

2. Material and methods

2.1. Study population and samples

A retrospective study was conducted on 152 respiratory samples stored at the Fondazione IRCCS Policlicnico San Matteo, Pavia, and Fondazione Cà Granda Ospedale Maggiore, Policlinico, Milano between 2011 and 2013 from as many neonates (age <30 days). Samples included 149 (97.9%) nasopharyngeal aspirates, 1 (0.7%) nasal swab, 1 (0.7%) bronchoalveolar lavage, and 1 (0.7%) tracheal aspirate.

This retrospective study was approved by the institutional review board (IRB) of both centres. In addition, the study was performed according to guidelines of the IRB on the use of biologic specimens for scientific purposes in keeping with Italian law (art.13 D.Lgs 196/2003).

2.2. FilmArray RP

The FilmArray RP detected the following agents: human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), influenza A virus, influenza A/H1 virus, influenza A/H3 virus, influenza A/H1N1pdm09 virus, influenza virus B, human adenovirus (hAdV), human parainfluenza virus types 1–4 (hPIV1-4), human rhinovirus (HRV), human enterovirus (HEV), human coronavirus (hCoV)-OC43, hCoV-229E, hCoV-NL63, hCoV-HKU1, bocavirus (hBoV), *Bordetella*

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Table 1
Combination of multiple viruses identified using FilmArray RP in archived samples from neonatal patients.

Viral combination detected	
hRSV + HRV/HEV	9
hRSV + hCoV	5
hRSV + influenza A virus	2
hRSV + bocavirus	1
hRSV + HRV/EV + hCoV-OC43	1
hRSV + HRV/EV + *M. pneumoniae*	1
hRSV + hCoV-OC43 + influenza A virus	2
hRSV + HRV/HEV + hPIV1 + hBoV	1
hRSV + HRV/HEV + influenza B virus + hBoV	1
hRSV + HRV/HEV + hAdV + hBoV	1
HRV/HEV + hCoV	2
HRV/HEV + influenza A virus	1
HRV/HEV + hPIV3	1
HRV/HEV + hBoV	1
HRV/HEV + hPIV3 + hBoV	1
HRV/HEV + influenza B virus + hBoV	1
HRV/HEV + hAdV + hBoV	1
HRV/HEV + hPIV3 + hAdV + hMPV + hBoV	1
hCoV-NL63 + hPIV3	1
hCoV-OC43 + hMPV	1
Influenza A virus + hBoV	1
Influenza A virus + hAdV + hBoV	1
Total	37

pertussis, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*. The FilmArray RP pouch system contains dried reagents for all the steps needed for extraction, PCR amplification, and detection of the respiratory viruses listed above. The pouch was rehydrated under negative pressure with 1 mL of molecular reagents grade water. Samples (250 μL) were diluted in 500-μL sample buffer, 300 μL of which was injected into the sample port. The results were available in about 1 hour after placing the pouch in the FilmArray Instrument.

2.3. Laboratory-developed real-time reverse transcriptase polymerase chain reaction (RT-PCR) and Seegene Seeplex RV15® detection kit

Seventy-two samples were previously tested using a panel of laboratory-developed real-time RT-PCR or real-time PCR (Piralla et al., 2009, 2011) able to detect and quantify the following viruses: influenza virus A and B, including subtype determination (WHO, 2009); hPIV3 (Hu et al., 2005); hRSV types A and B (Perkins et al., 2005); hCoV types OC43, 229E, NL63, and HKU1 (Dare et al., 2007); hMPV (Kuypers et al., 2005); and HRV (Lu et al., 2008).

Eighty samples were previously tested using the Seeplex RV15® detection kit (Seegene, Inc., Seoul, South Korea), which is designed for the simultaneous detection of 15 respiratory viruses, including influenza A virus, influenza B virus, hRSV type A and B, hAdV, hMPV, hPIV1-4, HRV, HEV, hCoV-229E/NL63, and hCoV-OC43/HKU1, and hBoV.

2.4. Statistical analyses

Positive percentage agreement and negative percentage agreement were assessed for FilmArray RP assay with respect to the both laboratory-developed real-time RT-PCR and the Seegene Seeplex RV15® assay.

3. Results

3.1. Detection of respiratory viruses by FilmArray RP

Overall, 121/152 (79.6%) samples were positive for at least 1 respiratory virus, while 31/152 (20.4%) were negative. In 84/121 (69.4%) positive samples, single-virus infections were shown. In detail, HRV/HEV (n = 33), hRSV (n = 32), hCoV (n = 7), hPIV1-4 (n = 5), influenza A virus (n = 3), influenza B virus (n = 2), and hBoV (n = 1) were detected. In the remaining 37/121 (30.6%) samples, multiple viruses were identified (Table 1). In detail, 25/37 (67.6%) samples were double positive, and 12 (32.4%) were positive for at least 3 viruses (Table 1). In addition, 1 sample was positive for *Mycoplasma pneumoniae*.

3.2. FilmArray RP versus laboratory-developed real-time RT-PCR panel

The real-time RT-PCR panel detected at least 1 respiratory virus in 63/72 (87.5%) samples while 9/72 (12.5%) were negative. FilmArray RP results were concordant with results obtained using laboratory-developed real-time RT-PCR in 68 of 72 (94.4%) respiratory samples tested while, in 4/72 (5.6%) samples, were discordant (Table 2). Among discordant results, the FilmArray RP assay detected additional viruses as follows: 2 HRV/HEV and 2 hBoV (Tables 3 and 4). However, hBoV was not included in the panel of agents detected by the laboratory-developed real-time RT-PCR panel. No *B. pertussis*, *C. pneumoniae*, or *M. pneumoniae* (not included in the laboratory-developed real-time RT-PCR panel) were detected in samples.

Quantitative results were available for the samples previously analyzed by laboratory-developed real-time RT-PCR for hRSV, HRV, and hCoV. All these viruses were identified also by the FilmArray RP assay independently from the viral load of the samples. The viral load range was 7.7×10^3–7.4×10^6 copies/mL of respiratory sample for hRSV (n = 32), 1.8×10^3–7.4×10^6 copies/mL of respiratory sample for HRV (n = 29), and 5.0×10^1–1.9×10^6 copies/mL of respiratory sample for hCoV (n = 8).

3.3. FilmArray RP versus Seegene Seeplex RV15®

Overall, 58/80 (72.5%) samples were positive for at least 1 respiratory virus, while 22/80 (27.5%) were negative with the Seegene Seeplex RV15® assay. FilmArray RP results were concordant in 70/80 (87.5%) respiratory samples tested using Seegene Seeplex RV15®, while 10/80 (12.5%) samples were discordant (Table 2). In 3/80 (3.8%) samples, there was no agreement between FilmArray RP assay and Seegene Seeplex RV15®. Among the 3 discordant results, 1 was in single-virus infections, and 2, in multiple-virus infection: FilmArray missed 1 HRV/HEV, 1 influenza virus B, and 1 hPIV3 infection (Tables 3 and 4). In 7/80 (8.7%) samples, the FilmArray RP assay detected an additional pathogen. In detail, 3 HRV/HEV, 3 hBoV (all in multiple-virus infections), 1 simultaneous detection of HRV/HEV and hBoV, and finally in 1 sample *M. pneumoniae* were detected concomitantly with hRSV and HRV/HEV (Tables 3 and 4). No *B. pertussis* or *C. pneumoniae* (not included in the Seegene Seeplex RV15® assay) were detected.

Table 2
Overall concordant, discordant, and agreement results for laboratory-developed real-time RT-PCR panel and Seegene Seeplex RV15® assay compared with FilmArray.

Assay	Laboratory-developed RT-PCR				Positive percent agreement	Negative percent agreement	Seegene Seeplex RV15®				Positive percent agreement	Negative percent agreement
	+/+	−/−	+/−	−/+			+/+	−/−	+/−	−/+		
FilmArray RP	59	9	0	4	93.7	100	49	21	3	7	87.5	87.5

Table 3
Detection results for laboratory-developed real-time RT-PCR panel and Seegene Seeplex RV15® assay compared with FilmArray.

Viruses	Laboratory-developed RT-PCR versus FilmArray						Seegene Seeplex RV15® versus FilmArray					
	Single infection			Multiple infection			Single infection			Multiple infection		
	+/+	−/+	+/−	+/+	−/+	+/−	+/+	−/+	+/−	+/+	−/+	+/−
hRSV	22	0	0	12	0	0	10	0	0	13	0	0
HRV/HEV	17	0	0	10	**2**	0	14	**2**	**1**	9	**2**	0
hCoV	4	0	0	3	0	0	3	0	0	8	0	0
hPIV1-4	4	0	0	1	0	0	1	0	0	3	0	**1**
hMPV	0	0	0	0	0	0	0	0	0	2	0	0
Influenza A virus	0	0	0	1	0	0	3	0	0	6	0	0
Influenza B virus	0	0	0	0	0	0	2	0	0	1	0	**1**
hAdV	0	0	0	0	0	0	0	0	0	3	0	0
hBoV[a]	NA	NA	NA	NA	NA	NA	1	0	0	4	**4**	0

Discordant results are reported in bold.
NA = not applicable.
[a] Bocavirus was tested with FilmArray RP assay and Seegene Seeplex RV15®.

4. Discussion

Respiratory infections are 1 of the leading causes of death in high-risk patients including newborns (Collins et al., 2012; Nair et al., 2013). Moreover, admission frequency for severe acute lower respiratory syndrome is more than 3 times higher in neonates with respect to older children (Nair et al., 2013). Detection of respiratory viruses by multiplex PCR has been described as an important tool for the accurate identification of pathogens causing respiratory syndromes (Mahony, 2008; Caliendo, 2011). Recently, the FilmArray RP assay was compared with different commercial molecular assays in pediatric and non-pediatric patients and showed good concordance of results (Babady et al., 2012; Hayden et al., 2012; Pierce et al., 2012; Rand et al., 2011). In this study, we carried out a retrospective analysis to evaluate FilmArray RP performance in neonates admitted to neonatal care units.

Virus detection with the FilmArray RP assay was comparable with respect to the laboratory-developed real-time PCR and Seegene Seeplex RV15® detection systems in almost all samples analyzed. Agreement was the same within samples with a single virus as well as those with multiple viruses. Only a few samples showed discordant results. However, the FilmArray RP assay identified additional viruses such as HRV/HEV and bocavirus, which were missed by both laboratory-developed real-time PCR and Seegene Seeplex RV15® as also reported by Pierce et al. (2012). In our series, this finding is likely due to 2 different biases related to the methods used to test the samples. In detail, the FilmArray RP assay simultaneously detects HRV and HEV, while the laboratory-developed real-time RT-PCR is specific for HRV. Hence, the FilmArray RP–positive samples could contain HEV besides HRV. hBoV was not included in the laboratory-developed real-time PCR panel. The decreased performance for hAdV detection reported by others (Pierce et al., 2012) was not fully evidenced in our series due to the limited number of hAdV positive samples. Finally, 2 major disadvantages were highlighted: i) the single-sample run that restricts the use of FilmArray to numerically limited routines and ii) the qualitative results obtained. On the other hand, the advantages of the FilmArray RP are the rapid detection of respiratory viruses with a turnaround time of 1 hour for each specimen, the wide number of pathogens detected in a single assay, and the reduced hands-on time. In addition, the FilmArray assay also includes hBoV and 3 bacteria (*B. pertussis*, *C. pneumoniae*, and *M. pneumoniae*) not included in other respiratory detection panels.

In conclusion, strong concordance between the laboratory-developed real-time RT-PCR, Seegene Seeplex RV15® and FilmArray RP results was observed. Simplicity and random access characterize FilmArray RP rendering it a valid choice for urgent pathogen detection in high-risk patients groups.

Table 4
Differences between laboratory-developed real-time RT-PCR panel or Seegene Seeplex RV15® results and FilmArray results.

FilmArray RP assay	Laboratory-developed real-time RT-PCR	Seegene Seeplex RV15®
hRSV, HRV/HEV	hRSV	
hRSV, HRV/HEV	hRSV	
HRV/HEV, hBoV	HRV	
RSV, hBoV	hRSV	
hRSV, HRV, influenza B virus, hBoV		hRSV, HRV, influenza B virus
Negative		HEV
HRV, PIV-3, hBoV		HRV, hPIV3
hRSV, HRV, *M. pneumoniae*		hRSV, HRV
HRV/HEV, influenza A/H1N1pdm09 virus		Influenza A virus
HRV/HEV		Negative
HRV/HEV		HRV, influenza B virus
Influenza B virus, HRV/HEV, hBoV		Influenza B virus
HRV/HEV		hPIV3
Influenza A/H1N1psm09 virus, hBoV		Influenza A virus

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